PB82-154808

The Ontogeny of Resistance Adaptation and Metabolic Compensation to Salinity and Temperature by the Caridean Shrimp, 'Palaemonetes pugio', and Modification by Sublethal Zinc Exposure

Texas A and M Univ.
College Station

Prepared for

Environmental Research Lab.
Gulf Breeze, FL

Jan 82

U.S. Department of Commerce
National Technical Information Service
NTIS

ERL,GB 127

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before compl

1. REPORT NO.	2.	
EPA-600/D-82-140	BOOK ARTICLE	PB82-154808

4. TITLE AND SUBTITLE	5. REPORT DATE
The Ontogeny of Resistance Adaptation And Metabolic Compensation to Salinity and Temperature by the Caridean Shrimp, Palaemonetes pugio, and Modification by Sublethal Zinc Exposure	January 1982
	6. PERFORMING ORGANIZATION CODE 026051
7. AUTHOR(S) Charles L. McKenney and Jerry M. Neff	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Biology Texas A&M University College Station, Texas	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. ID075-04890
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Office of Research and Development Environmental Research Laboratory Gulf Breeze, Florida 32561	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/04

15. SUPPLEMENTARY NOTES
Ref: Biological Monitoring of Marine Pollutants, Academic Press, Inc., NY pp. 205-240

16. ABSTRACT

Changing patterns in survival and metabolic rates were measured during the complete larval development of the estuarine shrimp, Palaemonetes pugio, reared from hatch through metamorphosis in 9 combinations of salinity (7-31 °/ooS) and temperature (20-30°C), both with and without exposure to a sublethal zinc concentration (0.23 ppm Zn). While early larval stages are extremely tolerant of a broad range of salinities and temperatures, late larval stages, beginning with the second to the last larval stage, are more sensitive to tmeperature than early larval stages.

The most pronounced influence of sublethal zinc exposure on metabolic rates of developing larvae was a depression of the respiratory rates of the first stage, second from the last and the last larval stage under conditions of moderate to high temperatures in high salinities and in low salinity-temperature conditions.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	Unclassified	38
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/D-82-140
Book Article

THE ONTOGENY OF RESISTANCE ADAPTATION AND METABOLIC COMPENSATION TO SALINITY AND TEMPERATURE BY THE CARIDEAN SHRIMP, *PALAEMONETES PUGIO*, AND MODIFICATION BY SUBLETHAL ZINC EXPOSURE

Charles L. McKenney, Jr.[1]
Jerry M. Neff[2]

Department of Biology
Texas A&M University
College Station, Texas

INTRODUCTION

Organisms occupying estuaries are those that through the process of natural selection have developed the capacity to resist the characteristically fluctuating salinity and temperature conditions of estuarine waters (Vernberg and Vernberg, 1972a; Lockwood, 1976). A number of recent studies, however, indicate that exposure to concentrations of heavy metals which prove sublethal under optimal salinity-temperature conditions for survival may reduce both salinity and temperature resistance capacities of estuarine organisms (Vernberg and Vernberg, 1972b; Vernberg *et al.*, 1973, 1974; Jones, 1973, 1975a; Rosenberg and Costlow, 1976; McKenney and Costlow, 1977; McKenney and Neff, 1979).

Adaptations of organisms to estuarine conditions are not, however, confined to mere tolerance of variations in temperature and salinity. While the basic mechanisms which allow for compensatory physiological adaptations to temperature (Hazel and Prosser, 1974; Somero and Hochachka, 1976)

[1]*present address: United States Environmental Protection Agency, Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida.*
[2]*present address: Battelle, William F. Clapp Laboratories, Inc., 397 Washington St., Duxbury, Massachusetts.*

and salinity (Giles, 1975; Lockwood, 1976) may be extremely complex, the results may be accompanied by alterations and, upon completion of acclimation, stabilization of energy utilized for metabolic maintenance, (Kinne, 1964, 1971; Schlieper, 1971; Pandian, 1975; Bayne *et al.*, 1976).

While numerous studies have demonstrated metabolic compensation by marine and estuarine invertebrates to both temperature (for a review see Kinne, 1970) and salinity (for a review see Kinne, 1971) acting individually, the combined effects of both factors acting simultaneously on the metabolism of marine invertebrates are poorly understood. Recognizing that early developmental stages of marine invertebrates are generally more sensitive to environmental variations than are adults, several studies have examined metabolic adjustments to both salinity and temperature in larval and juvenile stages of marine crustaceans (Engel and Angelovic, 1968; Vernberg *et al.*, 1973, 1974; Nelson *et al.*, 1977). However, no information exists on metabolic compensation to both factors throughout total larval development: nor has consideration been given to potential alterations of such compensatory mechanisms during larval development following exposure to heavy metals.

In an earlier paper, we reported an area of optimal salinity-temperature conditions within which viability of *Palaemonetes pugio* larvae through metamorphosis was maximal and outside of which total survival through complete larval development of this estuarine shrimp was reduced. Furthermore, resistance adaptations of developing larvae to salinity and temperature were progressively restricted with exposure to increasing sublethal zinc concentrations (McKenney and Neff, 1979). The objectives of the present study were to examine the ontogeny of possible resistance and metabolic capacity adaptations to salinity and temperature through the complete larval development of the caridean shrimp, *Palaemonetes pugio*, and to examine any disruptions in these responses by sublethal zinc exposure.

MATERIALS AND METHODS

Ovigerous female *Palaemonetes pugio* (Holthuis) were collected from East Galveston Bay, Texas at salinities ranging between 19 and 27 °/₀₀ and were transported to the laboratory in College Station, Texas. In the laboratory adults were maintained at a salinity of 19 °/₀₀ and at the experimental temperatures of (20°, 25°, or 30°C) approximating water temperatures at the collection site. Upon hatching,

larvae to be reared in the intermediate salinity (17 or 19 °/₀₀) were transferred directly to the various test media. To avoid osmotic shock, freshly hatched larvae to be reared in either lower or higher salinities were tranferred at three hour intervals in graded steps of 6-7 °/₀₀ S from the original intermediate salinity to the appropriate experimental salinity.

Larvae were individually reared in isolation in compartmented plastic boxes in an approximate liquid volume of 50 ml. All larvae were fed daily an abundance of freshly hatched *Artemia salina* nauplii in an approximately equal portion (2 nauplii per ml). Every third day larvae were transferred to an acid-cleaned compartmented box containing freshly prepared media of the appropriate salinity-temperature-zinc condition.

Desired salinities were obtained by diluting 35 °/₀₀ S seawater (prepared from Instant Ocean Synthetic Sea Salts containing an undetectable 20 ppb Zn) with distilled water. Measurements of the pH of the various experimental media reflected those of natural estuarine water at the respective salinities. Experimental temperatures were held within ±0.5°C of the desired temperature in constant-temperature cabinets that also maintained a 12 h light: 12 h dark photoperiod. Zinc solutions were prepared every third day from a primary stock solution of 1 g Zn++ per liter prepared from $ZnCl_2$. Analysis of zinc solutions by atomic absorption spectrophotometry revealed no significant difference between nominal and actual zinc concentrations nor significant loss of zinc from the compartmented boxes after 96 h at any of the experimental temperatures (McKenney and Neff, 1979).

The experimental design was a 3x3x2 factorial with salinities of 7, 19, and 31 °/₀₀; constant temperatures of 20°, 25°, and 30°C; and nominal zinc concentrations of 0.00 (unexposed controls) and 0.25 ppm Zn. For experiments on survival of each larval stage, four replicates of 12 larvae each were reared at each of the 18 salinity-temperature-zinc conditions. Additional larvae were individually reared at the various salinity-temperature-zinc conditions for use in the metabolic studies. Daily records were kept on mortality of all larvae and the molting frequency of each individual.

Larval oxygen consumption was determined by individually sealing five larvae from each salinity-temperature-zinc condition in 5-ml all-glass syringes of fully oxygenated media at the acclimation salinity. The volumes of media in the syringe varied from 1-5 ml dependent on the size of the larva. To avoid any variation in respiratory activity due to molt cycle, oxygen consumption of larvae was measured on days representative of their intermolt period. After a 3-4 hour period, a 75-100 µl sample of the fluid in the syringe was

injected into a Radiometer BMS 3 Mk2 micro blood gas analyzer with attached PHM71 Mk2 Acid-Base Analyzer and a direct measurement recorded for partial pressure of oxygen. The larva was then removed from the respirometer, briefly rinsed in distilled water, and placed in an oven to dry at 60°C for 48 hours. The dry weight of each larva was subsequently determined on a Mettler UM7 ultra-microbalance to the nearest 0.01 µg. Differences in partial pressures of oxygen between the values of control respirometers (syringes with no animal) and respirometers containing a larva were attributed to larval uptake of oxygen. These values were corrected for weight of larva and time in respirometer, and weight-specific oxygen consumption rates (µg O_2 mg^{-1} dry weight h^{-1}) were calculated.

For both oxygen consumption rates and arcsine-transformed percentage survival data of each developmental stage, multiple linear regression analysis was performed on three separate models with an increasing order of complexity. The choice of the most appropriate model was made by a step-down analysis which indicated whether decreasing complexity significantly increased the residual mean square. Since for both sets of data the most complex model proved to be the "best model" (significantly reduced the residual mean square), the results of this model will be presented here. A more detailed description of the results of each regression model and the step-down analysis is available in McKenney (1979).

To further aid in the interpretation of these data, separate regression equations were produced at each level of the two discrete independent variables (zinc exposure level and developmental stage) and employed to generate response surface curves at a range of levels of the two continuous independent variables (salinity and temperature) (Box, 1954; Box and Youle, 1955; Myers, 1971; Alderdice, 1972). Hence, for each developmental stage both with and without sublethal zinc exposure, the survival and respiration rates were expressed as the quadratic function of salinity and temperature by the two factor second-order polynomial equation:

$$\hat{Y} = b_0x_0 + b_1x_1 + b_{11}x_1^2 + b_2x_2 + b_{22}x_2^2 + b_{12}x_1x_2 \qquad (1)$$

where $\hat{Y}$ = the estimated weight-specific oxygen consumption rate or arcsine-transformed survival percentage; x_0 = the intercept; x_1 and x_2 = linear effect of salinity (Sal) and temperature (Temp), respectively; x_1^2 and x_2^2 = the quadratic effect of salinity (Sal^2) and temperature ($Temp^2$), respectively; x_1x_2 = the linear x linear interaction between salinity and temperatures (Sal x Temp); and b_0, b_1, etc. = the regression coefficients.

RESULTS

Survival of Individual Larval Stages

Percent survival of the various larval stages of *Palaemonetes pugio* varied from 31% to 100% over a range of salinity-temperature conditions with and without exposure to 0.25 ppm Zn (Table 1). Multiple linear regression analysis indicated the dominant effects of salinity and temperature on larval survival. Furthermore, the larval stage and zinc-stage interaction influenced the survival of developing larvae (Table 2). Pooled data of zinc-exposed and control larval survival analyzed by stage in a Duncan's Multiple Range Test indicated a significant reduction of survival percentages of later larval stages from those of the early larval stages and significantly lower survival in the last larval stage. Moreover, a number of interactions significantly modified the survival of developing larvae, including in order of importance: salinity, stage, and zinc; salinity, temperature, stage, and zinc; salinity and stage; temperature and stage; salinity, temperature, and stage; temperature and zinc; and temperature, stage, and zinc (Table 2).

Since the number of larval stages varied with salinity-temperature-zinc conditions, response surfaces were developed from quadratic salinity-temperature equations for categories of early and late larval stages (larval stages I-IV and second from the last, next to the last, and final larval stages, respectively). Regression coefficients for each separate equation are provided in the appendix of McKenney (1979). The statistical analyses for these curves are summarized in Table 3.

In the absence of additional zinc the survival of the first four larval stages was little affected by salinity and temperature (Figs. 1 and 2; Table 3). Exposure to 0.25 ppm Zn, however, significantly reduced the survival of first stage larvae at low salinity-temperature conditions. Viability of third stage larvae was affected by a salinity-temperature interaction in the presence of zinc, but in this case survival was most reduced at low temperature and high salinity conditions.

In general, late larval stages were more sensitive to temperature than early larval stages (Figs. 3 and 4; Table 3). Salinity and the salinity-temperature interaction initially reduced survival of developing larvae at the next to the last stage and in the last larval stage reduced survival under conditions of high temperature and high salinity. Zinc addition had the greatest effect on the survival of the last

TABLE 1. Percent survival of the various larval stages of *Palaemonetes pugio* over a range of salinity and temperature conditions when reared with and without the presence of 0.25 ppm Zn throughout the total developmental period.

Temperature (°C)		20			25			30		
Salinity (ppt)		7	19	31	7	19	31	7	19	31
Larval Stage	Zn (ppm)									
I	0.00	100	100	98	98	100	100	99	99	100
	0.25	31	100	100	100	100	100	96	100	100
II	0.00	98	100	98	100	98	94	100	100	100
	0.25	94	100	96	100	100	96	98	99	99
III	0.00	98	100	98	100	100	98	96	100	97
	0.25	100	100	91	100	100	98	97	100	99
IV	0.00	100	98	100	98	100	93	96	99	94
	0.25	100	100	94	100	100	100	99	97	99
V	0.00	100	100	98	100	98	97	92	94	91
	0.25	100	100	97	96	100	98	92	96	97
VI	0.00	100	100	100	98	100	100	94	92	68
	0.25	100	100	100	100	98	98	94	95	96
VII	0.00	98	100	100	98	98	100	98	100	64
	0.25	100	100	97	98	100	98	88	90	74
VIII	0.00	100	100	98	98	100	100			
	0.25	100	100	95	100	100	100			
IX	0.00	100	100	100	100	100	100			
	0.25	100	100	97	100	100	98			
X	0.00	100	93							
	0.25	100	97							
XI	0.00		97							
	0.25	100	96							
XII	0.00		94							
	0.25		92							
XIII	0.00									
	0.25		83							

TABLE 2. Multiple linear regression analysis of the effect of developmental stage, sublethal zinc exposure, salinity, and temperature on survival of developing *Palaemonetes pugio* larvae. Model terms follow those described in Equation (1). Source: source of variation; DF: degrees of freedom; MS: mean square; F value: ratio of treatment mean square to error mean square; PRF: probability of a larger F value. *Significant, **Highly significant.

Source	DF	MS	F value	PR>F
Correctional total	503	99.280		
Model	83	331.072	6.19	.0001**
Stg	6	214.124	4.00	.0007**
Zn	1	333.632	6.24	.0129**
Stg x Zn	6	192.775	3.61	.0017**
Sal	1	819.666	15.33	.0001**
Sal x Sal	1	550.384	10.29	.0014**
Temp	1	1789.912	33.47	.0001**
Temp x Temp	1	1810.905	33.87	.0001**
Sal x Temp	1	5.199	0.10	.7553
Sal x Stg	6	255.703	4.78	.0001**
Sal x Zn	1	41.424	0.77	.3793
Temp x Zn	1	319.210	5.97	.0150*
Temp x Stg	6	192.429	3.60	.0017**
Sal x Stg x Zn	6	525.803	9.83	.0001**
Temp x Stg x Zn	6	113.885	2.13	.0490*
Sal x Sal x Stg	6	355.664	6.65	.0001**
Sal x Sal x Zn	1	0.694	0.01	.9094
Temp x Temp x Stg	6	249.330	4.46	.0001*
Temp x Temp x Zn	1	226.954	4.24	.0400*
Sal x Temp x Stg	6	202.436	3.86	.0009**
Sal x Temp x Zn	1	162.544	3.04	.0820
Sal x Sal x Stg X Zn	6	95.557	1.79	.1002
Temp x Temp x Stg x Zn	6	75.486	1.41	.2086
Sal x Temp x Stg x Zn	6	444.710	8.32	.0001**
Error	420	53.473		

Table 3. Summary of the statistical analysis on the survival of each developmental stage of Palaemonetes pugio as influenced by the quadratic function of salinity (S) and temperature (T) and modifications by continuous zinc exposure. LI, LII, LIII, and LIV-first through the fourth larval form; SL, NL, and LL-second from the last, next to the last, and final larval form.

Developmental Stage	Zn (ppm)	Marked Effect P<.10	Significant Effect P<.05	Highly Significant Effect P<.005	Multiple Correlation
LI	0.00				.297
	0.25		S^2, T^2	S, T, SxT	.858**
LII	0.00	T, T^2			.487
	0.25				.335
LIII	0.00	S^2			.435
	0.25	S^2	SxT		.577*
LIV	0.00				.505
	0.25	T	T^2		.507
SL	0.00	T	T^2		.627*
	0.25	T	T^2, SxT		.717**
NL	0.00		S, T, T^2, SxT		.789**
	0.25	SxT	T, T^2		.564*
LL	0.00	T, T^2, SxT	S, S^2		.752**
	0.25	SxT	S^2	T, T^2	.819**

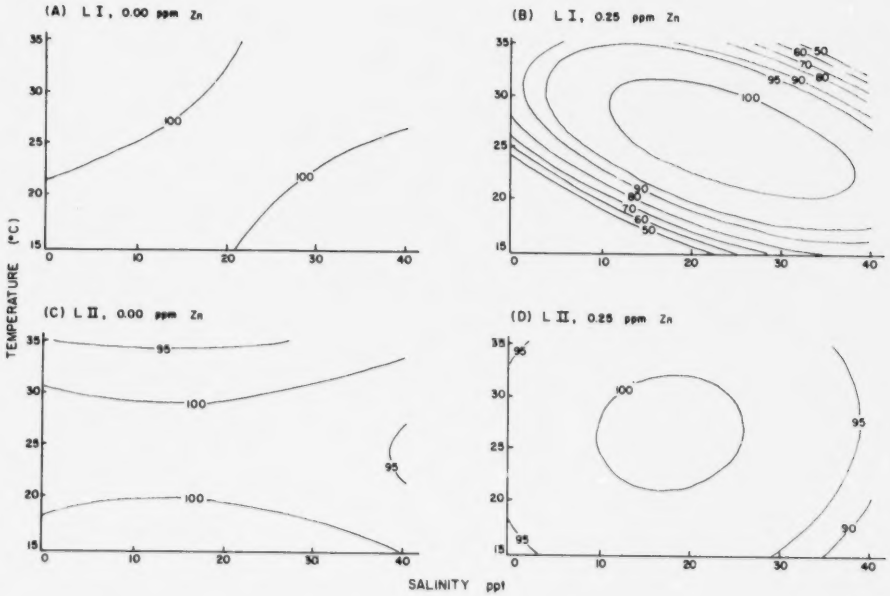

FIGURE 1. Estimation of percent survival of the first and second larval stages of Palaemonetes pugio *based on fitted response to observed survival at 9 combinations of salinity and temperature both with and without exposure to 0.25 ppm Zn. LI-first larval stage. LII-second larval stage.*

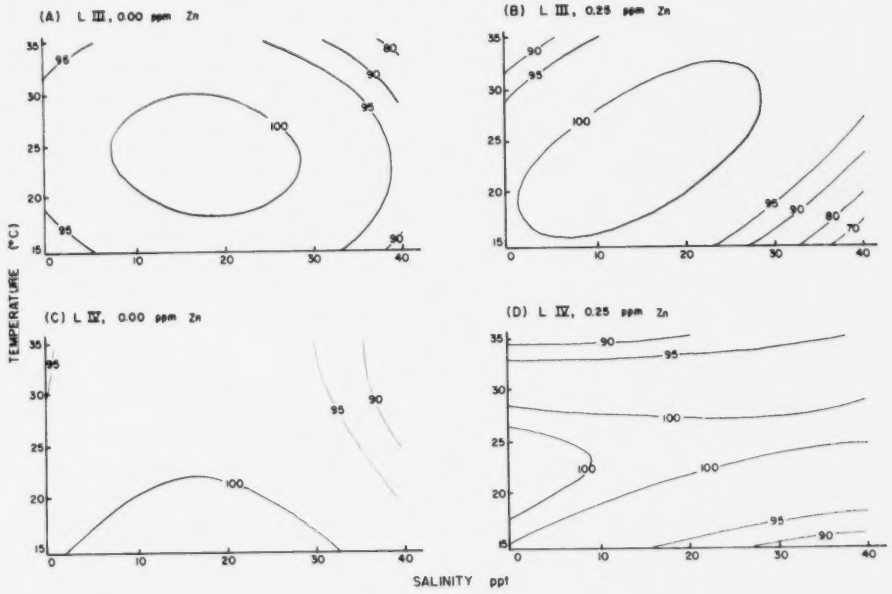

FIGURE 2. Estimation of percent survival of the third and fourth larval stages of Palaemonetes pugio *based on fitted response to observed survival at 9 combinations of salinity and temperature both with and without exposure to 0.25 ppm Zn. LIII-third larval stage. LIV-fourth larval stage.*

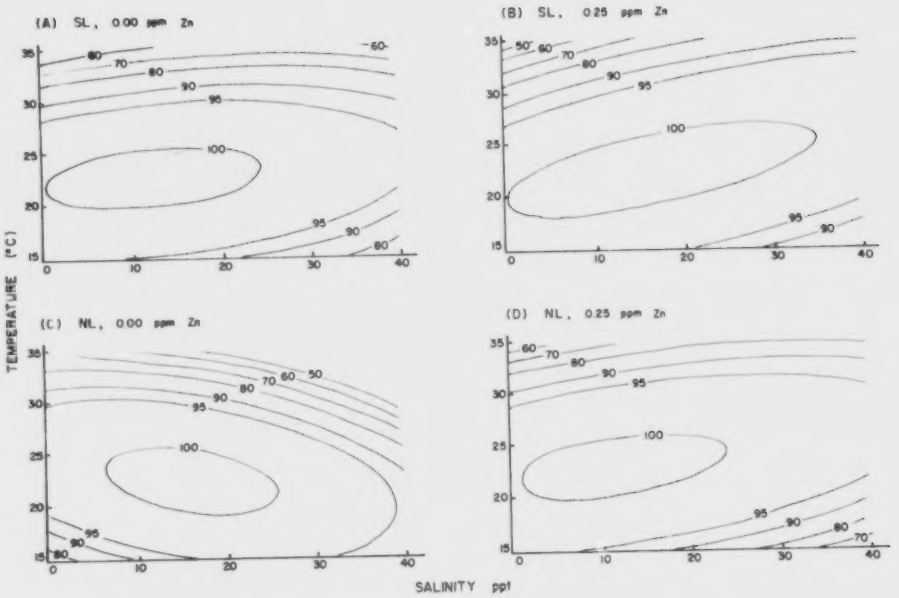

FIGURE 3. Estimation of percent survival of the second to the last larval stage and next to to the last larval stage of Palaemonetes pugio *based on fitted response to observed survival at 9 combinations of salinity and temperature both with and without exposure to 0.25 ppm Zn. SL-second from the last larval stage. NL-next to the last larval stage.*

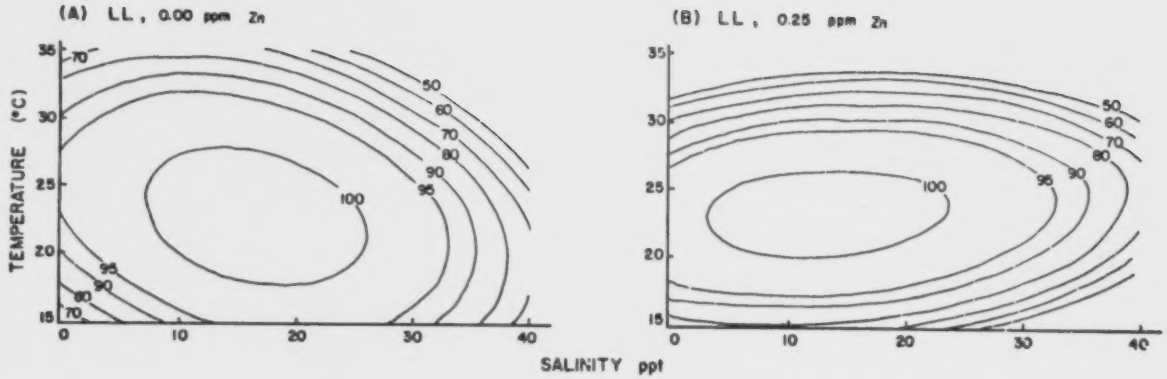

FIGURE 4. Estimation of percent survival of the last larval stage of Palaemonetes pugio *based on fitted response to observed survival at 9 combinations of salinity and temperature both with and without exposure to 0.25 ppm Zn. LL-last larval stage.*

larval stage by reducing the larval tolerance to temperature.

Oxygen Consumption

Oxygen consumption rates of the various larval stages of *Palaemonetes pugio* varied from 2.85 to 10.16 μg O_2 mg^{-1} dry weight h^{-1} when larvae were reared at a range of salinity-temperature conditions both with and without sublethal zinc exposure (Table 4). Multiple linear regression analysis demonstrated that oxygen consumption of developing larvae was not only significantly influenced by salinity and temperature, but that the quadratic function of salinity and temperature significantly modified larval uptake of oxygen dependent on stage of development and a zinc-stage interaction (Table 5). A Duncan's Multiple Range Test by stage of the pooled data for zinc-exposed and unexposed larvae suggested a significantly (alpha level = .05) lower oxygen consumption rate among the last three larval stages and the postlarvae. In addition, a number of interactions significantly influenced the rate of oxygen uptake by developing larvae, including in order of importance: temperature and stage; salinity, temperature and stage; salinity and stage; salinity, temperature, stage, and zinc; and salinity, stage and zinc (Table 5).

By using separate quadratic salinity-temperature equations for each stage and zinc condition, response-surface curves were generated for each stage-zinc condition over a range of salinity-temperature conditions. Regression coefficients for each separate equation are provided in the appendix of McKenney (1979). A summary of the analysis of variance of these equations is given in Table 6. All multiple correlations were significant at the .05 alpha level.

First stage larvae increased their rate of oxygen consumption with an increase in temperature to approximately 25°C; above this temperature, salinity interacted with temperature to produce increasing respiration rates at higher temperatures when coupled with a decrease in salinity (Fig. 5A). Sublethal zinc exposure exaggerated this salinity-temperature interaction in first stage larvae (Fig. 5B), such that it became more evident at lower temperatures and higher salinities to the point that above 25°C a lowering of the salinity dominated over temperature to increase the uptake of oxygen. Furthermore, at higher temperature-salinity conditions oxygen consumption rates were depressed. No such interaction was evident in the metabolic rates of second-stage larvae (Fig. 5C). However, second-stage larvae subjected to a zinc concentration of 0.25 ppm Zn exhibited both a significant

Table 4. Weight-specific oxygen consumption (µm mg^{-1} hr^{-1}) for the various larval stages of *Palaemonetes pugio* over a range of salinity and temperature conditions when measured with and without the presence of a sublethal zinc concentration (0.25 ppm Zn) throughout development.

Temperature (°C)		20			25			30		
Salinity (ppt)		7	19	31	7	19	31	7	19	31
Larval Stage	Zn (ppm)									
I	0.00	4.23±.08	3.31±.06	3.17±.12	6.22±.30	5.62±.70	6.40±.29	10.16±.72	6.15±.16	6.18±.88
	0.25	4.37±.16	3.62±.12	3.97±.03	7.27±.33	5.86±.31	5.84±.13	8.56±.51	6.47±.83	3.98±.53
II	0.00	3.38±.03	4.05±.07	3.97±.25	5.94±.35	5.67±.25	4.48±.29	8.57±.37	4.91±.35	8.08±.34
	0.25	5.00±.24	3.87±.32	3.89±.14	6.96±.37	5.31±.32	5.14±.23	7.18±.47	5.71±.65	8.84±.14
III	0.00	5.12±.13	3.62±.29	4.00±.07	5.59±.25	5.15±.24	5.36±.22	6.49±.48	6.03±.50	6.52±.23
	0.25	4.51±.21	3.89±.21	4.17±.16	5.64±.37	5.17±.18	5.71±.11	6.58±.25	7.98±.55	7.69±.40
IV	0.00	4.69±.08	2.85±.20	3.59±.23	5.79±.23	5.60±.34	5.37±.27	5.47±.15	5.11±.24	5.37±.12
	0.25	5.27±.37	3.14±.27	3.60±.09	5.18±.19	6.25±.26	5.08±.26	4.90±.25	4.61±.24	4.71±.34
V	0.00	5.55±.23	3.85±.27	3.49±.08	5.87±.62	5.12±.18	5.37±.12	5.58±.20	4.47±.22	4.91±.23
	0.25	5.80±.28	4.01±.13	3.41±.04	5.80±.32	5.34±.34	5.07±.19	5.62±.13	4.55±.21	3.59±.33
VI	0.00	5.57±.19	3.90±.22	3.55±.12	5.40±.28	4.37±.09	6.20±.23	5.26±.32	3.49±.14	4.81±.35
	0.25	5.92±.09	3.81±.19	4.37±.24	5.70±.15	5.38±.31	5.29±.21	4.78±.32	4.78±.32	4.45±.32
VII	0.00	5.23±.17	4.77±.14	4.33±.44	4.96±.22	4.08±.25	4.21±.32	5.32±.30	4.56±.85	5.27±.32
	0.25	5.15±.53	3.74±.19	3.83±.08	4.66±.29	3.83±.20	5.21±.09	5.07±.27	5.47±.16	3.01±.32
VIII	0.00	4.68±.21	4.12±.25	4.16±.22	4.37±.27	3.21±.07	4.34±.34			
	0.25	4.72±.51	3.33±.12	4.03±.25	4.60±.17	3.76±.39	4.43±.41			
IX	0.00	4.18±.04	4.03±.21	3.88±.10	5.03±.43	4.09±.25	3.31±.18			
	0.25	4.02±.26	3.83±.15	3.53±.12	4.93±.16	4.10±.15	4.09±.11			
X	0.00	4.41±.24		3.16±.14			3.90±.10			
	0.25	4.69±.39		3.36±.22			4.82±.15			
XI	0.00			3.55±.05			3.62±.28			
	0.25			3.15±.23			3.83±.17			
XII	0.00			3.05±.09						
	0.25			3.67±.11			3.36±.06			
XIII	0.00									
	0.25			3.52±.22						
Post-Larva	0.00	4.08±.21	3.54±.17	2.86±.29	4.62±.29	4.51±.26	5.14±.34	5.01±.15	5.07±.23	4.53±.79
	0.25	3.7±.29	4.03±.33	3.30±.15	5.23±.26	4.43±.16	5.23±.19	5.05±.42	4.83±.28	3.88±.31

TABLE 5. Multiple linear regression analysis of the effects of developmental stage, sublethal zinc exposure, salinity, and temperature on the oxygen consumption of developing *Palaemonetes pugio* larvae. Model terms follow those described in Equation (1). Source: source of variation; DF: degrees of freedom; MS: mean square; F value: ration of treatment mean square to error mean square; PR>F: Probability of a larger F value. *Significant: **Highly Significant.

Source	DF	MS	F value	PR F
Corrected total	650	2.0358		
Model	95	10.2196	16.07	.0001**
Stg	7	6.4295	10.11	.0001**
Zn	1	1.5212	2.39	.1224
Stg x Zn	7	0.7116	1.12	.3488
Sal	1	12.9415	20.36	.0001**
Sal x Sal	1	3.8436	6.05	.0142*
Temp	1	14.7513	23.21	.0001**
Temp x Temp	1	0.6972	1.10	.2954
Sal x Temp	1	0.0000	0.00	.9950
Sal x Stg	7	2.8473	4.48	.0001**
Sal x Zn	1	1.8857	2.97	.0856
Temp x Zn	1	1.4670	2.31	.1293
Temp x Stg	7	6.2099	9.77	.0001**
Sal x Stg x Zn	7	1.9709	3.10	.0034**
Temp x Stg x Zn	7	0.5885	0.93	.4867
Sal x Sal x Stg	7	0.7653	1.20	.2980
Sal x Sal x Zn	1	1.0886	1.71	.1912
Temp x Temp x Stg	7	4.9310	7.76	.0001**
Temp x Temp x Zn	1	0.0128	0.02	.8873
Sal x Temp x Stg	7	5.0030	7.87	.0001**
Sal x Temp x Zn	1	0.5275	0.83	.3627
Sal x Sal x Stg x Zn	7	0.6182	0.97	.4510
Temp x Temp x Stg x Zn	7	0.5118	0.81	.5847
Sal x Temp x Stg x Zn	7	17.3479	3.90	.0004**
Error	555	0.6357		

TABLE 6. Summary of the statistical analysis on the oxygen consumption of each developmental stage of *Palaemonetes pugio* as influenced by the quadratic function of salinity (S) and temperature (T) and modifications by continual sublethal zinc exposure. LI, LII, LII, LIV- first through the fourth larval form; SL, NL, and LL- second from the last, next to the last, and final larval form; and PL- postlarva. *P<.05, ** P<.005.

Developmental Stage	Zn (ppm)	Marked Effect P .10	Significant Effect P .05	Highly Significant Effect P .005	Multiple Correlation
LI	0.00	S^2, T, SxT			.800**
	0.25		S	T,T^2,SxT	.873**
LII	0.00		S^2		.810**
	0.25			S,S^2, SxT	.847**
LIII	0.00	SxT	S^2		.840**
	0.25				.900**
LIV	0.00	SxT	S^2		.805**
	0.25			T,T^2	.724**
SL	0.00	S	T,T^2		.780**
	0.25		SxT		.767**
NL	0.00	T,T^2			.680**
	0.25	T^2			.675**
LL	0.00	SxT	S		.711**
	0.25		SxT		.800**
PL	0.00		T,T^2		.780**
	0.25			T,T^2	.648**

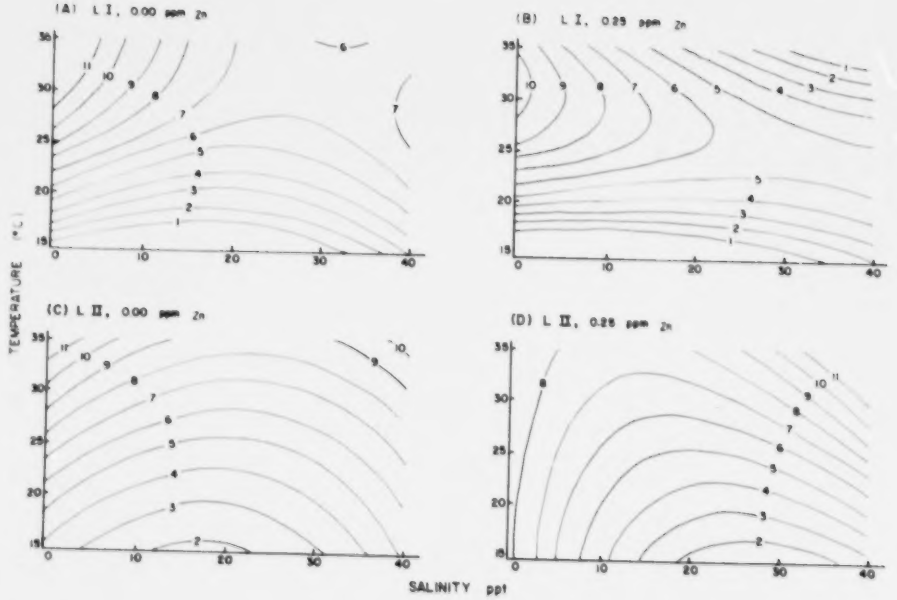

FIGURE 5. Estimation of the weight-specific oxygen consumption rates (µg O_2 mg^{-1} dry weight h^{-1}) of the first and second larval stages of Palaemonetes pugio *based on fitted response to observed rates at 9 combinations of salinity and temperature both with and without exposure to 0.25 ppm Zn. LI-first larval stage. LII-second larval stage.*

salinity-temperature interaction and a significant influence by salinity on oxygen consumption rates (Table 6 and Fig. 5D). At salinities above 20 °/₀₀ increased temperature accounted for the increase in oxygen consumption, while at lower salinities the influence of salinity dominated over elevated temperatures.

Salinity acted as the dominant factor affecting oxygen consumption of third-stage larvae by increasing the respiration rate at lower salinities (Fig. 6A). Sublethal exposure to zinc masked this relationship (Fig. 6B). Temperature exerted the grestest effect on the oxygen consumption of the fourth larval stage both with and without zinc exposure (Table 6 and Fig. 6C and 6D). Temperature increases to approximately 25°C produced an increase in the uptake of oxygen, at which point rates were stable (except at salinity extremes) to a temperature of about 31°C. Above this temperature rates were depressed by a temperature increase.

Weight-specific oxygen-consumption rates of late larval stages generally were less influenced by salinity and temperature (Figs. 7 and 8). Yet, the second to the last larval stage exhibited increased metabolic rates at temperatures higher and lower than 25°C and at lower salinities (Fig. 7A). In contrast, this larval stage when exposed to zinc increased oxygen uptake rates at low salinities and high temperatures (Fig. 7B). Unlike any other larval stage, contours for the estimated respiration rates of the next to the last larval stage demonstrated a salinity-temperature area at which oxygen uptake was minimal (Fig. 7C). Outside this area oxygen consumption increased, particularly at lower salinities. Oxygen uptake was reduced at low temperature and high salinity conditions for larvae exposed to 0.25 ppm Zn (Fig. 7D). At temperatures below 30°C, respiration rates for the last larval stage increased with decreasing salinities (Fig. 8A). Conversely, oxygen uptake by this stage when exposed to zinc was depressed and proved most sensitive to salinity at higher temperatures (Fig. 8B). A dramatic shift in salinity-temperature metabolic patterns occurred following completion of metamorphosis (Fig. 8C). Oxygen uptake of postlarvae was little affected by salinity, but principally influenced by temperature. Rates of oxygen consumption were depressed outside the approximate range of 25° to 30°C and more noticeably so for zinc-exposed larvae at higher temperatures and higher salinities (Fig. 8D).

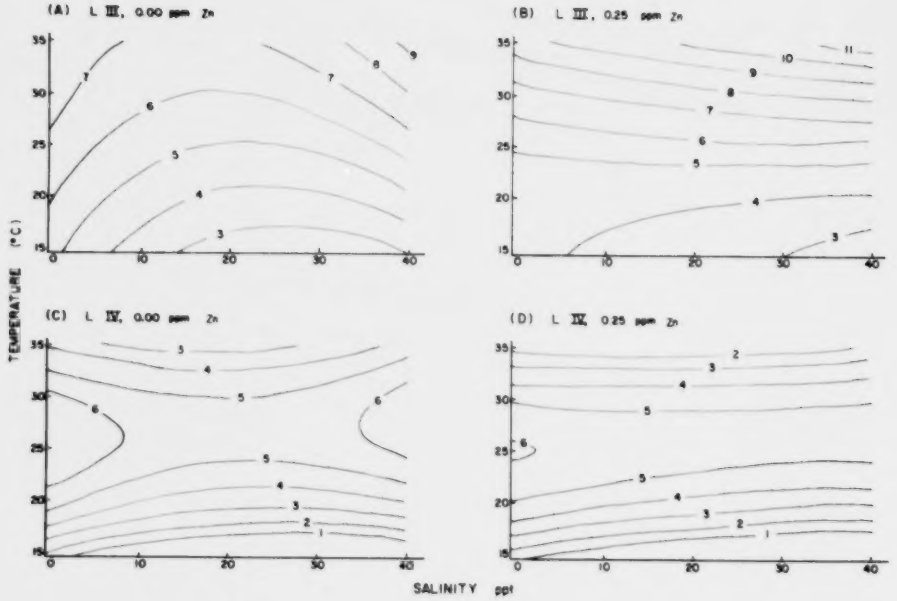

FIGURE 6. Estimation of the weight-specific oxygen consumption rates ($\mu gO_2\ mg^{-1}$ dry weight h^{-1}) of the third and fourth larval stages of Palaemonetes pugio *based on fitted response to observed rates at 9 combinations of salinity and temperature both with and without exposure to 0.25 ppm Zn. LIII-third larval stage. LIV-fourth larval stage.*

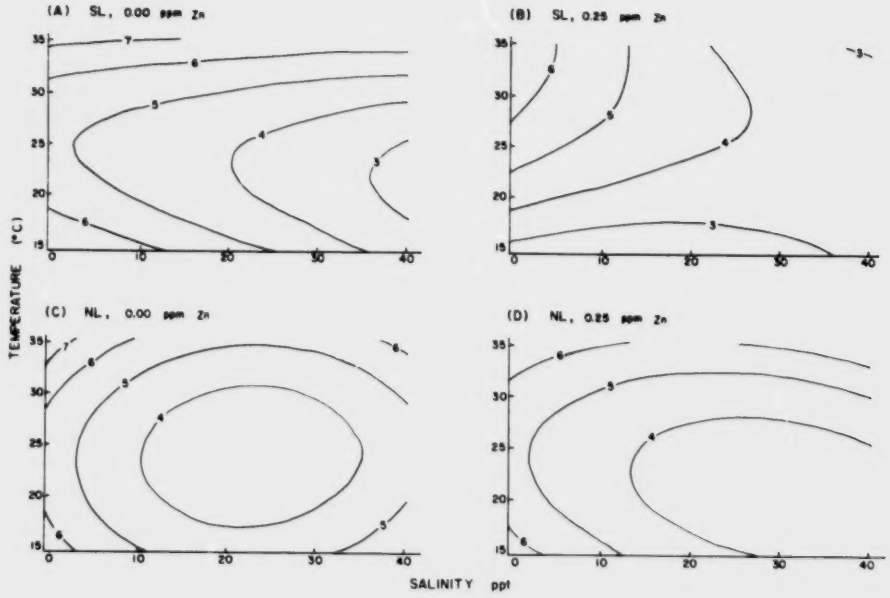

FIGURE 7. Estimation of the weight-specific oxygen consumption rates (µg O_2 mg^{-1} dry weight h^{-1}) of the second from the last and the next to the last larval stages of Palaemonetes pugio *based on fitted response to observed rates at 9 combinations of salinity and temperature both with and without exposure to 0.25 ppm Zn. SL-second from the last larval stage. NL-next to the last larval stage.*

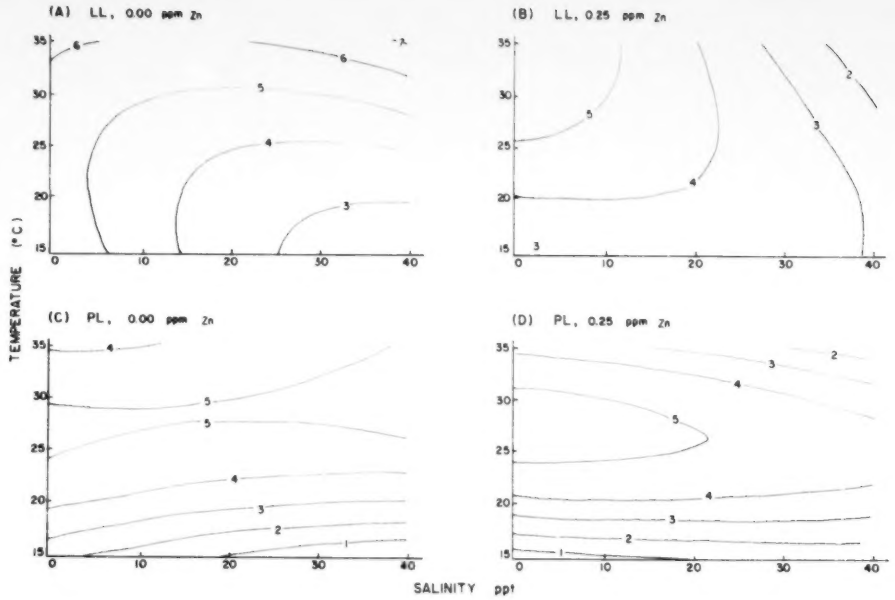

FIGURE 8. Estimation of the weight-specific oxygen consumption rates (µg O_2 mg^{-1} dry weight h^{-1}) of the last larval stage and postlarva of Palaemonetes pugio *based on fitted response to observed rates at 9 combinations of salinity and temperature both with and without exposure to 0.25 ppm Zn. LL-last larval stage. PL-postlarva.*

DISCUSSION

Survival of Individual Larval Stages

An earlier study demonstrated that not only were survival patterns of *Palaemonetes pugio* larvae throughout development modified by salinity and temperature conditions, but that exposure to concentrations of zinc which were sublethal at optimal salinity-temperature conditions reduced viability through complete larval development under stressful levels of salinity and temperature (McKenney and Neff, 1979). It appears, furthermore, that differential sensitivity to salinity, temperature, and/or zinc existed among the various individual developmental stages as indicated by the highly significant four factor interaciton term, salinity-temperature-zinc-stage. In the absence of additional zinc, early larval stages were extremely tolerant of a broad range of salinities and temperatures. Conversely, the late larval stages beginning with the second to the last larval stage appeared more sensitive to high temperatures, with this pattern shifting to reduced viability of the last larval stage at conditions of high temperature and high salinity. The greater sensitivity of the last larval stage to environmental stress may indicate the more delicate nature of this pre-metamorphic stage, possibly associated with a greater energetic demand prior to metamorphosis.

These changing salinity-temperature survival patterns during the ontogeny of *Palaemonetes pugio* were, in addition, modified by exposure to a sublethal zinc concentration and these alterations were stage dependent. The first and last larval stages were most sensitive to zinc exposure over a broad range of constant salinity and temperature conditions. In the presence of a sublethal zinc concentration, the first larval stage was significantly less resistant to low salinities at low temperatures. Following zinc exposure from hatch throughout complete larval development, the last larval stage became highly sensitive to temperature extremes prior to completion of metamorphosis.

With the additional stress of zinc exposure, the reduced viability of first-stage larvae to low salinity waters could be indicative of disruption of a less well developed system for coping with hypo-osmotic stress during this early developmental stage and, perhaps, heavy metal inhibition of enzyme systems associated with hypo-osmotic regulation (Schmidt-Nielsen, 1974; Bryan, 1976a, b). Although osmoregulatory abilities of larval *P. pugio* have not been investigated, postlarval *P. pugio* have been shown to be able to

regulate body fluids both hypo- and hyper-osmotically to environmental salinities outside their isosmotic salinity of 17 °/₀₀ (Roesijadi *et al.*, 1976). Such osmoregulatory abilities of estuarine organisms have been altered by sublethal heavy metal exposure (Thurberg *et al.*, 1973; Jones, 1975a, b; Inman and Lockwood, 1977).

These variations in resistance patterns to salinity and temperature during the larval development of *P. pugio* reflect the ontogeny of capacity for physiological adaptation to variable environmental conditions. As larval development progresses, the last two larval stages demonstrated reduced resistance to high salinities. This pattern suggests, therefore, an increased capacity for *P. pugio* to tolerate low salinities accompanied by a reduced capacity to tolerate high salinities. Such a developmental pattern of salinity tolerance in *P. pugio* agrees well with observations that freshly hatched larvae are released by ovigerous females found more abundantly in higher salinity waters (Wood, 1967), inferring limited hyper-regulatory abilities of early larval stages.

Respiration of Individual Larval Stages

As is common with poikilotherms, the oxygen consumption of early larval stages of *P. pugio* had a marked temperature dependence. Assuming oxygen consumption rates to be indirect measurements of metabolic rates, the metabolic rates of these early developmental stages were directly related to temperature. As temperature increased oxygen consumption rates were increased. Temperature dependent metabolism has been similarily reported for other decapod larvae (Vernberg and Costlow, 1966; Belman and Childress, 1973; Sastry and McCarthy, 1973; Nelson *et al.*, 1977; Schatzlein and Costlow, 1978). However, as larval development of *P pugio* continued, compensatory adjustments in metabolism allowed for a reduced temperature dependence in the oxygen consumption rates of the later larval stages. Upon completion of metamorphosis, oxygen consumption rates of postlarval *P. pugio* were highest at optimal temperatures for resistance and depressed at stressful high and low temperature extremes. Similar to these results, Sastry and Vargo (1977) reported that the earlier larval stages of *P. pugio* from Rhode Island were more metabolically sensitive to temperature fluctuations than later developmental stages.

The changing capacities of *Palaemonetes pugio* larvae to adjust metabolic rates to changes in temperature levels during ontogeny may reflect certain biochemical adjustments. Such biochemical mechanisms responsible for metabolic compensations to temperature include alterations in enzyme, substrate, and/or cofactor concentrations; variations in enzyme activities;

modulation of enzyme activities through the use of multiple enzyme forms (isozymes); and/or formation of enzyme variants with unique catalytic efficiencies (Hazel and Prosser, 1974; Somero and Hochachka, 1976). Although compensatory metabolic adjustments to temperature appear to be species-specific among marine crustacean larvae and reflective of individual tolerance ranges (Sastry and McCarthy, 1973; Sastry and Vargo, 1977), the results of recent biochemical studies on developing early life stages of several estuarine crustaceans suggest the application of similar adaptive mechanisms in the metabolic regulation of these larvae to changes in temperature, including observations of increased enzyme activities and increased isozyme concentrations later in larval development (Frank *et al.*, 1975; Morgan *et al.*, 1978; Sastry, 1978; Sastry and Ellington, 1978).

In addition to temperature-sensitive metabolic patterns, metabolic rates of developing *P. pugio* larvae were differentially altered by the salinity in which they were reared. These variations in respiratory rates were further modified by a salinity-temperature interaction for certain larval stages. Oxygen consumption of the first larval stage was progressively increased by salinities less than 20 $^{\circ}/_{\circ\circ}$, but only at temperatures above 25°C. Both lower and higher salinities than that which is isosmotic for postlarval and juvenile *P. pugio* (17 $^{\circ}/_{\circ\circ}$S; Roesijadi *et al.*, 1976) resulted in elevated respiratory rates of the second and third larval stages, particularly at higher temperatures. The dominant influence of temperature on metabolic patterns of the fourth larval stage masked the effects of salinity except at the optimal temperature of 25°C where respiratory rates were elevated at low and high salinity extremes. Unlike salinity-metabolic patterns of the early larval stages, respiratory rates of the last three larval stages were dominantly influenced by changes in salinity. At temperatures below approximately 30°C, oxygen consumption of the second to the last and the last larval stages was increased as salinity decreased. The metabolic pattern of the next to the last larval stage was unique; a temperature-salinity area existed within which oxygen consumption was minimum and outside this area metabolic rates were increased principally by salinities below 11 $^{\circ}/_{\circ\circ}$. Following completion of metamorphosis postlarval respiration was essentially unaffected by a change in salinity level.

A number of physiological mechanisms have been described which aid in the adaption of estuarine organisms to changes in salinity (i.e., volume regulation, isosmotic intracellular regulation and anisosmotic extracellular regulation) (Potts and Parry, 1964; Florkin and Schoffeniels, 1965; Prosser,

1973; Gilles, 1975; Lockwood, 1976; Smith, 1976). Several studies have described the functioning of some of these mechanisms in *P. pugio* (Roesijadi *et al.*, 1976; Lucu *et al.*, 1977). Measurements of the hemolymph osmotic concentrations of juvenile and adult *P. pugio* in salinities of 2 to 32 °/₀₀ indicated a highly developed hypo- and hyperosmotic regulation of body fluids as early as the postlarval stage (Roesijadi *et al.*, 1976). Both hemolymph sodium (Lucu *et al.*, 1977) and chloride (Roesijadi *et al.*, 1976) concentrations were maintained at relatively stable levels over this wide salinity range. Water permeability of *P. pugio* was greatest at the isosmotic salinity (17 °/₀₀ S) and was reduced at salinities associated with active osmoregulation (Roesijadi *et al.*, 1976). Unfortunately, similar studies describing osmoregulatory abilities of developing larval *P. pugio* have not been reported.

In general, the metabolic rate (oxygen consumption rate) of a euryhaline invertebrate will either (1) increase in subnormal salinities and/or decrease in supranormal salinities or (2) increase in sub- and supranormal salinities. The salinity-metabolic response of stenohaline invertebrates generally involves a decrease in oxygen consumption in sub- and supranormal salinities, while the respiratory rate of holeuryhaline invertebrates remains basically unaltered by salinity fluctuations (for a review of this subject see Kinne, 1971). For example, oxygen consumption of the euryhaline crab, *Panopeus herbstii*, was elevated in low salinities (Dimock and Groves, 1975). Respiration rates of the stenohaline osmoconformers, *Murex pomum* and *Strombus pugilis*, declined in stressful low salinities (Sander and Moore, 1978). Chronic exposures to salinities ranging from 3.5 to 32 °/₀₀ produced no appreciable variation in the routine metabolic rate of adult *Palaemonetes vulgaris* (McFarland and Pickens, 1965).

The respiratory responses of developing *Palaemonetes pugio* larvae to various salinity conditions basically followed the patterns described for euryhaline organisms. In general, oxygen consumption rates of early larval stages beyond the first stage were increased by both sub- and supranormal salinities, while those of the later larval stages were increased only by a decrease in salinity. The metabolic-salinity pattern of the postlarval stage was essentially unaffected by salinity and, therefore, followed the response pattern of a holeuryhaline organism, similar to that described for adult *P. vulgaris* (McFarland and Pickens, 1965). This ability to compensate metabolically to low salinity stress might be an important factor in the distribution of this species in upper reaches of estuaries (Bowler and Seidenberg,

1971; Thorp and Hoss, 1975).

Several explanations have been proposed regarding the physiological mechanisms that might be responsible for the observed alterations in respiratory rates with a change in salinity. While most authors have suggested that increased metabolic rates at salinities differing from the isomotic point are indicative of the increased energy cost due to osmoregulation, Potts (1954) produced a theoretical model indicating the metabolic cost of osmoregulation to be less than 2% of the basal metabolic rate. However, Shaw (1959) pointed out that the assumption that the outer surfaces of the organisms are semipermeable ("permeable to water but impermeable to salts"; Potts, 1954) was invalid. Recent evidence (as reviewed by Smith, 1976; Lockwood, 1976) suggests that the ability to vary surface permeability to both salts and water in response to changing salinities is an important adaptive osmoregulatory mechanism for a number of euryhaline invertebrates. Spaargaren (1974, 1975a, b), furthermore, has developed mathematical models, based on empirical evidence, to describe the effect of osmotic stress on the extracellular electrolyte concentration and energy relations in several species of crustaceans. These models suggest that the energy required for ionic regulation by *Carcinus meanas* is only a small part of the basal metabolism within the normal range of salinities experienced by the crab, but that at extreme salinities a substantial part of the total energy consumption is occupied in ionic regulation (Spaargaren, 1975b). More recently, Cameron (1978) calculated that the active Na^+ and Cl^- uptake system in the blue crab *Callinectes sapidus* would account for 20% of the total oxygen demands of the organisms in freshwater. Similarly, variations in respiratory rates of the shore crab *Carcinus meanas* to both salinity and temperature were related to changing patterns of osmoregulation (Taylor *et al.*, 1977).

The metabolic response of larvae to zinc was complicated by a significant zinc-salinity-temperature-stage interaction, indicating that zinc disrupted the metabolic patterns of certain developmental stages dependent on the salinity and temperature condition. The most pronounced influence of zinc exposure on the O_2 consumption of developing *Palaemonetes pugio* larvae was a depression of the rates in the first stage, second from the last and the last larval stage under conditions of moderate to high temperatures in high salinities and in low salinity-temperature conditions. This disruption of metabolic patterns of late larval stages continued following completion of metamorphosis with the respiratory rates of postlarvae depressed by zinc at high salinities and moderate to high temperatures. These alterations in metabolic

capacity are in agreement with reduced resistance adaptations previously described.

Zinc concentrations as high as 480 ppb have been measured in Corpus Christi Bay, Texas (Holmes *et al.*, 1974) and as high as 530 ppb in the Galveston Bay Estuarine System (Texas Water Quality Board, 1975). Since zinc concentrations reported from these estuaries are similar to those to which larvae were exposed in this study, speculation is appropriate. Disruption by heavy metal exposure of compensatory osmoregulatory adaptations have been described for a number of estuarine species (Schmidt-Nielsen, 1974; Jones, 1975a, b; Inman and Lockwood, 1977). Altered serum osmolalities in *Carcinas meanas* following exposure to cadmium was accompanied by a depressed oxygen consumption rate of gill-tissue (Thurberg *et al.*, 1973). Moreover, heavy metal toxicity is thought to be associated with inhibition of enzyme systems (Vallee and Ulmer, 1972; Bryan, 1976b). The depressed metabolic rates of certain larval stages of *Palaemonetes pugio*, along with reduced salinity resistance following zinc exposure, might reflect disruptions in osmoregulatory mechanisms in developing larvae resulting from enzyme inhibition by zinc.

It is interesting to note that as larval development of *Palaemonetes pugio* progresses through completion of metamorphosis, both reduced resistance capacities and reduced metabolic capacities following zinc exposure are associated to a greater extent with high salinities. The hyporegulation of body fluids, as reported for postlarval and juvenile *P. pugio* (Roesijadi *et al.*, 1976), is a relatively rare process among estuarine or marine invertebrates (Kinne, 1971; Gilles, 1975; Lockwood, 1976). The relatively greater metabolic sensitivity to zinc in higher salinities later in larval development may indicate less well developed physiological mechanisms for hyporegulation than for hyperregulation at this stage of development. Since adaptive capacities and the mechanisms involved are evolutionary products, then the ability to hyperregulate in freshwater during the evolutionary history of a genus might accompany a loss in ability to compensate to high salinities (Kinne, 1963; Hubschman, 1975). The changing ontogenetic adaptive capacities of *P. pugio* could be indicative of the phylogenetic migration of the genus *Palaemonetes* from life in marine waters to life in freshwater.

SUMMARY

Changing patterns in survival and metabolic rates were measured during the complete larval development of the estuarine shrimp, *Palaemonetes pugio*, reared from hatch through metamorphosis in 9 combinations of salinity (7-31 °/₀₀S) and temperature (20-30°C), both with and without exposure to a sublethal zinc concentration (0.25 ppm Zn). While early larval stages are extremely tolerant of a broad range of salinities and temperatures, late larval stages, beginning with the second to the last larval stage, are more sensitive to temperature than early larval stages. Furthermore, as larval development progresses, the last two larval stages demonstrate reduced resistance to high salinities. These changing salinity-temperature survival patterns during the ontogeny of *P. pugio* are modified by continuous sublethal zinc exposure and these alterations are stage dependent with the first and last larval stages being most sensitive to zinc. Upon exposure to zinc, the first larval stage is significantly less resistant to low salinities, especially at low temperatures, and the last larval stage, prior to completion of metamorphosis, becomes highly sensitive to high temperature extremes.

As with resistance, metabolic rates of developing *P. pugio* larvae were influenced by a salinity-temperature-stage-zinc interaction. Metabolic rates of early larval stages of *P. pugio* have a marked temperature dependence. As larval development continues, compensatory adjustments in metabolism allow for a reduced dependence of the respiration of the late larval stages to temperature. Upon completion of metamorphosis, metabolic rates are maximum in optimal temperatures for resistance and slightly depressed in stressful high and low temperature extremes. The metabolic responses of developing larvae to various salinity conditions basically follow the pattern described for euryhaline organisms. In general, metabolic rates of early larval stages beyond the first stage are increased by both sub- and supranormal salinities, while metabolic rates of late larval stages are increased by a decrease in salinity. The metabolic-salinity pattern of the postlarval stage is essentially unaffected by salinity and, therefore, follows the response pattern of a holeuryhaline organism.

The most pronounced influence of sublethal zinc exposure on metabolic rates of developing larvae was a depression of the respiratory rates of the first stage, second from the last and the last larval stage under conditions of moderate to high temperatures in high salinities and in low salinity-

temperature conditions. These alterations in metabolic capacity are in agreement with reduced resistance adaptations of *P. pugio* larvae reared under continuous exposure to a sublethal zinc concentration. Since adaptive capacities and the mechanisms involved are evolutionary products, it appears that changing ontogenetic adaptive capacities of *P. pugio* reflect phylogenetic migration from life in marine waters to life in freshwater with the ability to resist and compensate metabolically to less saline waters during the ontogeny of the species accompanying a loss in the ability to compensate to higher salinities under additional stressful conditions as imposed by sublethal zinc exposure.

ACKNOWLEDGMENTS

This study was supported by Grant No. ID075-04890 from the National Science Foundation I.D.O.E. Biological Effects Program to Jerry M. Neff. We gratefully acknowledge the patient assistance of Margaret Weirich during portions of this study.

LITERATURE CITED

Alderdice, D. F. 1972. Factor combinations: responses of marine poikilotherms to environmental factors acting in concert. In: Marine ecology. Vol. 1. Environmental factors, Part 3. pp. 1659-1772. Ed. by O. Kinne. London: Wiley-Interscience.

Bayne, B. L., J. Widdows, and R. H. Thompson. 1976. Physiological integrations. In: Marine mussels: their ecology and physiology. pp. 261-291. Ed. by B. L. Bayne. Cambridge: Cambridge University Press.

Belman, B. W., and J. J. Childress. 1973. Oxygen consumption of the larvae of the lobster *Cancer productus* (Randall). Comp. Biochem. Physiol. 44A, 821-828.

Bowler, M. W., and A. J. Seidenberg. 1971. Salinity tolerances of the prawns, *Palaemonetes vulgaris* and *P. pugio* and its relationship to the distribution of these species in nature. Va. J. Sci. 22, 94.

Box, G. E. P. 1954. The exploration and exploitation of response surfaces: Some general considerations and examples. Biometrics 10. 16-60.

Box, G. E. P., and P. V. Youle. 1955. The exploration and exploitation of response surfaces: An example of the link between the fitted surfaces and the basic mechanism of the system. Biometrics 11, 287-323.

Bryan, G. W. 1976a. Some aspects of heavy metal tolerance in aquatic organisms. In: Effects of pollutants on aquatic organisms. pp. 7-34. Ed. by A. P. M. Lockwood. Cambridge: Cambridge University Press.

Bryan, G. W. 1976b. Heavy metal contamination in the sea. In: Marine pollution. pp. 185-302. Ed. by R. Johnston. London: Academic Press.

Cameron, J. N. 1978. NaCl balance in blue crabs, *Callinectes sapidus*, in fresh water. J. Comp. Physiol. 123, 127-135.

Dimock, R. V., Jr., and K. H. Groves. 1975. Interaction of temperature and salinity on oxygen consumption of the estuarine crab. *Panopeus herbstii*. Mar. Biol. 33, 301-308.

Engel, D. W., and J. W. Angelovic. 1968. The influence of salinity and temperature upon the respiration of brine shrimp nauplii. Comp. Biochem. Physiol. 26. 749-752.

Florkin, M., and E. Schoffeniels. 1965. Euryhalinity and the concept of physiological radiation. In: Studies in comparative biochemistry. pp. 6-40. Ed. by K. A. Munday Oxford: Pergamon Press.

Frank, J. R., S. D. Sulkin, and R. P. Morgan, II. 1975. Biochemical changes during larval development of the xanthid crab, *Rhithropanopeus harrisii*. I. Protein, total lipid, alkaline phosphatase, and glutamic oxaloacetic transaminase. Mar. Biol. 32, 105-111.

Gilles, R. 1975. Mechanisms of ion and osmoregulation. In: Marine ecology. Vol. II. Physiological mechanisms, Part 1. pp. 259-347. Ed. by O. Kinne. London: Wiley-Interscience.

Hazel, J., and C. L. Prosser. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev. 54, 620-677.

Holmes, C. W., E. A. Slade, and C. J. McLerran. 1974. Migration and redistribution of zinc and cadmium in marine estuarine system. Envir. Sci. Technol. 8, 255-259.

Hubschman, J. H. 1975. Larval development of the freshwater shrimp *Palaemonetes kadiakensis* Rathburn under osmotic stress. Physiol. Zool. 48, 96-107.

Inman, C. B. E., and A. P. M. Lockwood. 1977. Some effects of methyl-mercury and lindane on sodium regeneration in the amphipod *Gammarus duebeni* during changes in the salinity of its medium. Comp. Biochem. Physiol. 58C. 67-75.

Jones, M. B. 1973. Influence of salinity and temperature on the toxicity of mercury to marine and brackish water isopods (Crustacea). Estuar. Cstl. Mar. Sci. 1, 425-431.

Jones, M. B. 1975a. Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine and estuarine isopods (Crustacea). Mar. Biol. 30, 13-20.

Jones, M. B. 1975b. Effects of copper on survival and osmoregulation in marine brackish water isopods (Crustacea). In: Proceedings of the ninth European marine biology symposium. pp. 419-431. Ed. by H. Barnes Aberdeen: Aberdeen University Press.

Kinne, O. 1963. Adaptation, a primary mechanism of evolution In: Phylogeny and evolution of crustacea. pp. 27-50. Ed. by H. B. Whittington and W. D. I. Rolfe. Harvard: Spec. Publ. mus. Comp. Zool.

Kinne, O. 1964. The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature-salinity combinations. Oceanog. Mar. Biol. Ann. Rev. 2, 281-339.

Kinne, O. 1970. Temperature: animals-invertebrates. In: Marine ecology. Vol. 1 Environmental factors, Part 1. pp. 407-514. Ed. by O. Kinne. London: Wiley-Interscience.

Kinne, O. 1971. Salinity: animals-invertebrates. In: Marine ecology. Vol. 1. Environmental factors, Part 2. pp. 821-995. Ed. by O. Kinne. London: Wildy-Interscience.

Lockwood, A. P. M. 1976. Physiological adaptations to life in estuaries. In: Adaptation to environment. pp. 315-392. Ed. by R. C. Newell. London: Butterworths.

Lucu, C., G. Roesijadi, and J. W. Anderson. 1977. Sodium kinetics in the shrimp *Palaemonetes pugio*. I. Steady-state and nonsteady-state experiments. J. Comp. Physiol. 115, 195-206.

McFarland, W. N., and P. E. Pickens. 1965. The effects of season, temperature, and salinity on standard and active oxygen consumption of the grass shrimp, *Palaemonetes vulgaris* (Say). Can. J. Zool. 43. 571-585.

McKenney, C. L., Jr. 1979. Ecophysiological studies on the ontogeny of euryplasticity in the caridean shrimp, *Palaemonetes pugio* (Holthuis) and modifications by zinc, 217 p. Ph.D. dissertation, Texas A&M University.

McKenney, C. L., Jr., and J. D. Costlow, Jr. 1977. Interaction of temperature, salinity, and mercury on larval development of the xanthid crab, *Rhithropanopeus harrisii* (Gould). Amer. Zool. 17, 922.

McKenney, C. L., Jr. and J. M. Neff. 1979. Individual effects and interactions of salinity, temperature, and zinc on larval development of the grass shrimp *Palaemonetes pugio*. I. Survival and developmental duration through metamorphosis. Mar. Biol. 52, 177-188,

Morgan, R. P., E. Kramarsky, and S. D. Sulkin. 1978. Biochemical changes during larval development of the xanthid crab *Rhithropanopeus harrisii*. III. Isozyme changes during otogeny. Mar. Biol. 48, 223-226.

Myers, R. H. 1971. Response surface methodology, 246 pp. Boston: Allyn & Bacon, Inc.

Nelson, S. G., D. A. Armstrong, A. E. Knight, and H. W. Li. 1977. The effects of temperature and salinity on the metabolic rate of juvenile *Macrobrachium rosenbergii* (Crustacea: Palaemonidae). Comp. Biochem. Physiol. 56A, 533-537.

Pandian, T. J. 1975. Mechanisms of heterotrophy. In: Marine ecology. Vol. II. Physiological mechanisms, Part 1. pp. 61-250. Ed. by O. Kinne. London: Wiley-Interscience.

Potts, W. T. W. 1954. The energetics of osmotic regulation in brackish- and freshwater animals. J. Exp. Biol. 31, 618-630.

Potts, W. T. W., and G. Parry. 1964. Osmotic and ionic regulation in animals, 423 pp. Oxford: Pergamon Press.

Prosser, C. L. 1973. Comparative animal physiology, 996 pp. Philadelphia: Saunders.

Roesijadi, G., J. W. Anderson, S. R. Petrocelli, and C. S. Giam. 1976. Osmoregulation of the grass shrimp *Palaemonetes pugio* exposed to polychlorinated biphenyls (PCBs). I. Effect on chloride and osmotic concentrations and chloride and water-exchange kinetics. Mar. Biol. 38, 343-355.

Rosenberg, R., and J. D. Costlow, Jr. 1976. Synergistic effects of cadmium and salinity combined with constant and cycling temperature on the larval development of two estuarine crab species. Mar. Biol. 38, 291-303.

Sander, F., and E. A. Moore. 1978. Comparative respiration in the gastropods *Murex pomun* and *Strombus pugilis* at different temperatures and salinities. Comp. Biochem. Physiol. 60A, 99-105.

Sastry, A. N. 1978. Physiological adaptation of *Cancer irroratus* larvae to cyclic temperatures. In: Physiology and behaviour of marine organisms. pp. 57-65. Ed. by D. S. McLusky and A. J. Berry. Oxford: Pergamon Press.

Sastry, A. N., and W. R. Ellington. 1978. Lactate dehydrogenase activity during the larval development of *Cancer irroratus:* effect of constant and cyclic thermal regimes. Experientia 34, 308-309.

Sastry, A. N., and J. F. McCarthy. 1973. Diversity of metabolic adaptations in pelagic larval stages of two sympatric species of brachyuran crabs. Neth. J. Sea Res. 7, 434-446.

Sastry, A. N., and S. L. Vargo. 1977. Variations in the physiological responses of crustacean larvae to temperature. In: Physiological responses of marine biota to pollutants. pp. 401-425. Ed. by F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg. New York: Academic Press.

Schatzlein, F. C., and J. D. Costlow, Jr. 1978. Oxygen consumption of the larvae of the decapod crustaceans, *Emerita talpoida* (Say) and *Libinia emarginata* Leach. Comp. Biochem. Physiol. 61A, 441-450.

Schlieper, D. 1971. Physiology of brackish water. In: Biology of brackish water. pp. 211-350. Ed. by A. Remane and C. Schlieper. New York: Wiley-Interscience.

Schmidt-Nielsen, B. 1974. Osmoregulation: effect of salinity and heavy metals. Fedn. Proc. Fedn Am. Socs. Exp. Biol. 33, 2137-2146.

Shaw, J. 1959. Salt and water balance in the east African freshwater crab, *Potamon niloticus* (M. Edw.). J. Exp. Biol. 36, 157-176.

Smith, R. I. 1976. Apparent water-permeability variations and water exchange in crustaceans and annelids. In: Perspectives in experimental biology. Vol. 1. Zoology. pp. 17-24. Ed. by P. S. Davies. Oxford: Pergamon Press.

Somero, G. N., and P. W. Hochachka. 1976. Biochemical adaptations to temperature. In: Adaptation to environment. pp. 125-190. Ed. by R. C. Newell. London: Butterworths.

Spaargaren, D. H. 1974. A study on the adaptation of marine organisms to changing salinities with special reference to the shore crab, *Carinus maenas* (L). Comp. Biochem. Physiol. 47A, 499-512.

Spaargaren, D. H. 1975a. Energy relations in the ion regulation in three crustacean species. Comp. Biochem. Physiol. 51A, 543-548.

Spaargaren, D. H. 1975b. Heat production of the shore-crab *Carcinus maenas* (L). and its relation to osmotic stress. In: Proceedings of the ninth European marine biology symposium. pp. 475-482. Ed. by H. Barnes. Aberdeen:

Aberdeen University Press.

Taylor, E. W., P. J. Butler, and A. Al-Wassia. 1977. The effect of a decrease in salinity on respiration, osmoregulation and activity in the shore crab, *Carcinus maenas* (L.) at different acclimation temperatures. J. Comp. Physiol. 119, 155-170.

Texas Water Quality Board. 1975. Special report on Houston Ship Channel monitoring program. 9 pp. Report No. SR-3, District 7, mimeogr.

Thorp, J. H., and D. E. Hoss. 1975. Effects of salinity and cyclic temperatures on survival of two sympatric species of grass shrimp (*Palaemonetes*), and their relationship to natural distributions. J. Exp. Mar. Biol. Ecol. 18, 19-28.

Thurberg, R. P., M. A. Dawson, and R. S. Collier. 1973. Effects of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs. Mar. Biol. 23, 171-175.

Vallee, B. L., and D. D. Ulmer. 1972. Biochemical effects of mercury, cadmium, and lead. An. Rev. Biochem. 41, 91-128.

Vernberg, F. J., and J. D. Costlow, Jr. 1966. Studies on physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. IV. Oxygen consumption of larvae and young crabs reared in the laboratory. Physiol. Zool. 39, 36-52.

Vernberg, W. B., P. J. DeCoursey, and J. O'Hara. 1974. Multiple environmental factor effects on physiology and behaviour of the fiddler crab, *Uca pugilator*. In: Pollution and physiology of marine organisms. pp. 381-426. Ed. by F. J. Vernberg and W. B. Vernberg. New York: Academic Press.

Vernberg, W. B., P. J. DeCoursey, and W. J. Padgett. 1973. Synergistic effects of environmental variables on larvae of *Uca pugilator*. Mar. Biol. 22, 307-312.

Vernberg, W. B., and F. J. Vernberg. 1972a. Environmental physiology of marine animals. 346 pp. New York: Springer-Verlag.

Vernberg, W. B., and F. J. Vernberg. 1972b. The synergistic effect of temperature, salinity, and mercury on survival and metabolism of the adult fiddler crab, *Uca pugilator*. Fish. Bull. U.S. 70, 415-420.

Wood, C. E. 1967. Physioecology of the grass shrimp, *Palaemonetes pugio*, in the Galveston Bay estuarine system. Contrib. Mar. Sci. 12, 54-79.